the thin filament, whereas the dilated cardiomyopathy mutations (TnTR141W and TnTdeltaK210) accelerated the rate of Ca2+ dissociation from the thin filament. The Ca2+ sensitizing compound bepridil had no effect on the rate of Ca2+ dissociation in either thin filaments or myofibrils, but engineered mutations of TnC can accelerate or slow the rate. Finally, the alpha and beta isoforms of tropomyosin slowed and accelerated the rate of Ca2+ dissociation from the thin filament, respectively. Thus, multiple factors can modulate the rate of Ca2+ dissociation from the thin filament.

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Kinetics of Ca^{2+} Dissociation-Induced Structural Transitions of Cardiac Thin Filament

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Ca^{2+} induces structural transitions within the cardiac thin filament. The structural kinetics at the troponin-actin interface was investigated by Förster Resonance Energy Transfer (FRET) to understand the molecular basis underlying thin filament regulation. The kinetics of the Ca^{2+}-induced conformational changes at the cTnC N-domain, the cTnC-cTnI and the cTnI-actin interfaces were studied. The structural transition of the cTnC N-domain was examined by monitoring FRET between a donor (AEDANS) attached to one cysteine and an acceptor (DDPM) attached the other cysteine mutant of cTnC(13C/51C). The cTnC-cTnI interactions were investigated by monitoring the distance changes from cTnC(89C) to cTnI(151C) and cTnI(167C). Both cTnI(151C) and cTnC(167C) were labeled with AEDANS as FRET donor and cTnC(89C) was labeled with DDPM as the FRET acceptor. These two labeled cTnI mutants were also used to monitor Ca^{2+}-induced distance changes from cTnI residues 151 and 167 to the cysteine residue 374 of actin labeled with DABM as the FRET acceptor. Results from FRET Ca^{2+} titrations and stopped-flow kinetic measurements demonstrated that different structural transitions have different Ca^{2+} sensitivities and different Ca^{2+} dissociation-induced kinetics. Structural transitions involving the regulatory region and the mobile domain of cTnI occurred at fast kinetic rates, while the structural transitions involving transversal movement of the cTnI inhibitory region occurred at slow kinetic rates. Our results suggest a two-step deactivation of the thin filament upon Ca^{2+} dissociation. The first step may involve rapid binding of the mobile domain of cTnI to actin, which was kinetically coupled with the conformational change of cTnC N-domain and dissociation of the regulatory region of cTnI from the cTnC hydrophobic pocket. The second step involved the inhibitory region of cTnI switching its interacting from cTnC to actin. The latter processes may participate in regulating crossbridge kinetics.

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An Internal Domain of Beta Tropomyosin Increases Myofilament Calcium Sensitivity

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Tropomyosin (TM) is involved in calcium mediated muscle contraction and relaxation in the heart. Striated muscle alpha TM is the major isoform expressed in the heart. Expression of striated muscle beta TM in murine myocardium results in a decreased rate of relaxation and increased myofilament calcium sensitivity. Replacing the carboxyl terminus (amino acids 258-284) of alpha TM for beta TM (a troponin-T [TnT] binding region) results in decreased rates of contraction and relaxation in the heart and decreased myofilament calcium sensitivity. We hypothesized that the putative internal TnT binding domain (amino acids 175-190) of beta TM may be responsible for the increased myofilament calcium sensitivity observed when the entire beta TM is expressed in the heart. To test this hypothesis, we generated transgenic mice that express a chimeric TM containing beta TM amino acids 175-190 in the backbone of alpha TM (amino acids 1-174 and 191-284). These mice express 16% - 57% chimeric TM, and they do not develop cardiac hypertrophy or any other morphological changes. Physiological analysis shows these hearts exhibit systolic and diastolic dysfunction and a positive response to isoproterenol. Skinned fiber bundle analyses show a significant increase in myofilament calcium sensitivity. Biophysical studies demonstrate that the exchanged amino acids do not influence the flexibility of TM. This is the first study to demonstrate that a specific domain within TM can increase calcium sensitivity of the thin filament. Further, these results enhance the understanding of why TM mutations associated with familial hypertrophic cardiomyopathy also demonstrate increased myofilament sensitivity to calcium.

2595-Pos Board B565

The Assessment of Uncertainty in Measurement of Cholesterol: A Model of Calculation

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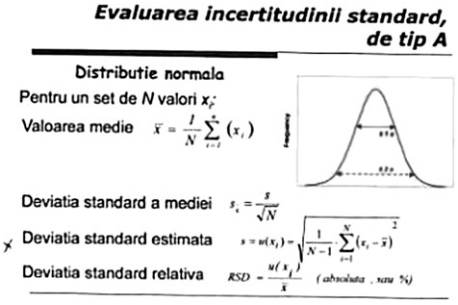
The objective of this work was to identify all the components of uncertainty in measurement of Cholesterol, undergoing a reasonable estimation of results in the acceptable method.
Material and Method:
For identification of the uncertainty in measurement of Cholesterol in laboratory, running Hitachi 912 Roche Analyzer, was necessary the Standard Cholesterol (ST).
Results:
Was calculated, in function of Standard Deviation (SD) and Student Factor (t), by estimation (SD * t), Compound Uncertainty of ST, assembling Uncertainty of A type and Uncertainty of B type, in value of 11 mg%.
Conclusion:
Budget of Uncertainty, in assessment of Cholesterol was established to a permitted error of 11% in normal range and under cut-off.



Microtubules & Microtubule-associated Proteins

2596-Pos Board B566

Tau Directly Inhibits The On-rate Of Kinesin, For Microtubules, During Transport

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Kinesin belongs to a class of microtubule (MT) based molecular motors, which can facilitate the intracellular transport of vesicle bound cargo and organelles throughout the cell. This vital function is especially pronounced in the neuron, where transport down exceedingly long processes, such as axons and dendrites, must be efficiently accomplished and cannot be explained be diffusion alone. Like most cellular processes, regulation is a fundamental aspect of kinesin mediated intracellular trafficking and to date many modes to regulate kinesin have been elicited. Recently the microtubule associated protein (MAP), tau, has been implicated in playing a central role in the regulation of kinesin mediated transport in the neuron. Tau has previously been shown to reduce the procesivity and attachment frequency of kinesin motors on MTs. Although it has been demonstrated that tau has a dramatic affect on kinesin based transport, the mechanism by which this occurs is presently unknown. Using stopped-flow rapid kinetics, we demonstrate that tau directly affects the on-rate of kinesin for MTs causing the motor to dissociate from the MT track. Because kinesin releases ADP at a greatly accelerated rate in the presence of MTs, we can effectively monitor the on-rate of kinesin for MTs by following the release of the fluorescent ADP analog mantADP. We demonstrate the on-rate of kinesin, for MTs, is reduced by tau in both a concentration and isoform specific manner.

2597-Pos Board B567

Synchrotron X-ray Scattering Study of the Effects of Microtubule-associated-protein (MAP) Tau on Interprotofilament and Intermicrotubule Interactions

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